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MATURATION OF THE ADRENAL MEDULLA: CATECHOLAMINE STORES IN NORMAL AND HYPERTENSIVE RATS

Earlier progress reports from this project detailed the biochemical maturation of the adrenal medulla in normotensive Wistar rats (NWR) as well as evaluating adrenal catecholamine synthesis, storage and release in adult spontaneously hypertensive rats (SHR), and therefore these results will not be discussed here. As described in progress report number 3, the properties of storage vesicles from SHR suggested that their responses to autonomic drugs might be different from those of NWR. Studies with reserpine have now been completed and will be published shortly (see publications list).

Reserpine administration resulted in a larger initial decline in adrenal catecholamines in spontaneously hypertensive rats (SHR) than in normotensive Wistar rats (NWR); the difference was eliminated by pretreatment with chlorisondamine. Reserpine also produced a larger increase in SHR catecholamines and dopamine β-hydroxylase several days later; chlorisondamine pretreatment did not prevent the increases, although it did slightly slow the increases. Vesicles from SHR or NWR incubated with reserpine in vitro demonstrated equivalent inhibition of $ATP\text{-}Mg^{2+}$-stimulated epinephrine uptake. Recovery of uptake was more rapid in SHR than in NWR after reserpine inhibition, and this was associated with a burst of new vesicle synthesis in the SHR; chlorisondamine pretreatment reduced the number of new, immature vesicles in reserpine-treated SHR. Both SHR and NWR secreted equal proportions of their adrenal catecholamine contents after nicotine administration. These data suggest that the sympatho-adrenal system of the SHR exhibits an enhanced reflex response to reserpine but that reserpine is equally effective in SHR and NWR in producing blockade of vesicular catecholamine transport; these alterations can affect markedly the actions of autonomic drugs in the SHR.

Similar studies are now underway with nicotine and chlorisondamine (a nicotinic blocking agent). In NWR, nicotine evokes a sympatho-adrenal discharge, resulting in catecholamine depletion, tyrosine hydroxylase induction, and formation of immature vesicles. Chlorisondamine blocks many of the effects of nicotine, but itself evokes some reflex activity. The effects of nicotine disappears upon chronic administration. We will evaluate in the near future whether the acute and chronic effects of nicotine and chlorisondamine are different in the SHR.

The above studies suggest a resemblance between the SHR and human labile hypertension - i.e., periods of low sympathetic activity coupled with hyper-responsiveness to reflex stresses. Preliminary results obtained with developing SHR indicate that, prior to 3 weeks of age (pre-

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hypertensive phase) basal sympathetic activity is high (resembling human essential hypertension); these studies will be continued with other sources of support.

An offshoot of this project has been the development of drugs with reserpine-like sympatholytic activity. An additional publication (see list) details further progress made in this area. Two amine uptake mechanisms appeared to operate in isolated adrenal medullary storage vesicles; one site had a high affinity for epinephrine (Km=30 μM) and low capacity (Umax=20 nmols/100 μg of endogenous catecholamines), while the other had a low affinity (Km=2 mM) and a higher capacity (Umax=130 nmols). The low affinity site was non-specific and did not display competitive inhibition by agents which affected the high affinity, stimulated transport system. The high affinity system was inhibited in a purely competitive fashion by a variety of indoleamines and phenethylamines, but the two classes of compounds displayed different structure-activity relationships. Substitution on the α-carbon decreased the abilities of indoleamines to inhibit stimulated epinephrine uptake, but enhanced activity of phenethylamines. Ring hydroxylation reduced, and methoxylation eliminated, the inhibitory activity of tryptamine, but the same substituents markedly enhanced the activity of phenethylamines. Studies of compounds with restricted side-chain conformation indicated that a condensed structure favored activity in indoleamines while an extended chain enhanced inhibition by phenethylamines. Linear alkylamines of 5 or 6 carbon length were also able to inhibit active epinephrine uptake. None of the agents inhibited the non-stimulated uptake of component of metaraminol, which uses primarily the low affinity system. These data suggest that while indoleamines and phenethylamines do compete with epinephrine for attachment to the high affinity transport site in the vesicle membrane, the point of interaction is probably solely at the locus which binds the amine nitrogen; the remainder of the two types of molecule probably bind to at least two different sites adjacent to the N-binding area.

Publications from this project:

1. Maturation of the adrenal medulla. I. Uptake and storage of amines in isolated vesicles of the rat. T.A. Slotkin, Biochem. Pharmacol. 22:2023-2032 (1973).

2. Maturation of the adrenal medulla. II. Content and properties of catecholamine storage vesicles of the rat. T.A. Slotkin, Biochem. Pharmacol. 22:2033-2044 (1973).

3. Binding of amines to purified adrenal medullary storage vesicle membranes. T.A. Slotkin and N. Kirshner, Biochem. Pharmacol. 22: 2492-2497 (1973).

4. Hypothetical model of catecholamine uptake into adrenal medullary vesicles. T.A. Slotkin, Life Sci. 13:675-683 (1973).

5. Reserpine-like effects of harmine on isolated adrenal medullary storage vesicles. H.O. Green and T.A. Slotkin, Mol. Pharmacol. 9:748-755 (1973).

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6. Secretion and recovery of catecholamines by the adrenal medulla. N. Kirshner and T.A. Slotkin, Biochem. Pharmacol., in press.

7. Drug-resistant effect of adenine nucleotides and magnesium on catecholamine efflux from isolated adrenal medullary storage vesicles. T.A. Slotkin and H.O. Green, Biochem. Pharmacol., in press.

8. Maturation of the adrenal medulla. III. Practical and theoretical considerations of age-dependent alterations in kinetics of incorporation of catechol- and non-catecholamines. T.A. Slotkin, Biochem. Pharmacol., in press.

9. Adrenal medullary storage vesicles of the spontaneously hypertensive rat. T.A. Slotkin and H.O. Green, Biochem. Pharmacol., in press.

10. Structure-activity relationships for the reserpine-like actions of derivatives of beta-carboline. T.A. Slotkin, Life Sci., in press.

11. Effects of reserpine on the adrenal medulla of the spontaneously hypertensive rat. T.A. Slotkin, Brit. J. Pharmacol., in press.

12. Inhibition of epinephrine and metaraminol uptake into adrenal medullary vesicles by aralkyl- and alkylamines. T.A. Slotkin, T.R. Anderson, F.J. Seidler and C. Lau, Biochem. Pharmacol., in press.

13. Acute and chronic effects of nicotine on synthesis and storage of catecholamines in the rat adrenal medulla. T.A. Slotkin and F.J. Seidler, in preparation.

14. Sympatho-adrenal stimulation and inhibition by acute and chronic chlorisondamine administration to rats. T.A. Slotkin, S.M. Schanberg, F.J. Seidler, C. Lau, J. Bartolome, J. Cooke, Biochem. Pharmacol., submitted.

Abstracts:

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1. Uptake and storage of amines in isolated adrenal medullary vesicles of developing rats. T.A. Slotkin, Fed. Proc. 32:783Abs. (1973).

2. Maturation of adrenal catecholamine storage vesicles of the rat. T.A. Slotkin, Pharmacologist 15:210 (1973).

3. Adrenal medullary vesicles of hypertensive rats. T.A. Slotkin and H.O. Green, Clin. Res. 22:13A (1974).

4. Effects of tryptamines on epinephrine uptake into adrenal medullary vesicles. T.A. Slotkin, F.J. Seidler and M.D. Abou-Donia, Pharmacologist 16:257 (1974).

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